Alboatisins A−C, *ent*-Atisene Diterpenoids from *Isodon albopilosus*

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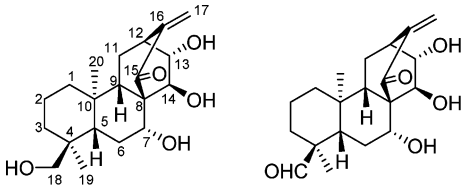
Three biogenetically interesting *ent*-kaurane-derived metabolites, alboatisins A−C (**1**−**3**), featuring a C-13-oxygenated *ent*-atisane skeleton, have been isolated from the aerial parts of *Isodon albopilosus*. Their structures were determined on the basis of spectroscopic evidence. Compound **2** exhibited cytotoxicity against A549 and HT-29 human cancer cells, with IC_{50} values of 8.3 and 7.9 μM, respectively.

Isodon (formerly *Rabdosia*) includes about 150 species and is one of the largest genera of the plant family Labiatae. It has attracted considerable attention as a rich source of new diterpenes with diverse structures and interesting biological properties.[1−4] For the past 30 years, as part of a search for novel natural products as useful leads for the discovery of therapeutic agents to treat cancer, more than 50 *Isodon* species from mainland China were investigated phytochemically. About 500 new diterpenoids with diverse skeleta have been isolated and characterized, including some *ent*-kauranoids with potential antitumor activity and low toxicity,[3,4] such as eriocalyxin B,[5] oridonin,[6] and ponicidin.[6] In the present work, we have reinvestigated the aerial parts of *Isodon albopilosus*.[7] Nine *ent*-kauranoids, albopilosins B−J, were isolated from this species in an earlier publication.[8] In this paper, we report the isolation, structure elucidation, and biological evaluation of three new *ent*-atisane diterpenoids, called alboatisins A−C (**1**−**3**).



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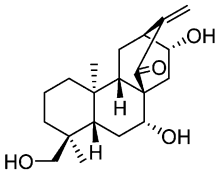

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Alboatisin A (**1**) was isolated as a white powder ($[\alpha]_D^{19}$ −18.6; *c* 0.13, CH_3OH) that gave a $[M + Na]^+$ ion at *m*/*z* 373.1984 in the HRESIMS, consistent with a molecular formula of $C_{20}H_{30}O_5$ (calcd 373.1990), requiring six sites of unsaturation. The IR spectrum of **1** showed bands characteristic of hydroxyl groups and an α,β-unsaturated ketone group (ν_{max} 3424, 1711, and 1632 cm^{-1}). Twenty resonances (5 × C; 6 × CH; 7 × CH_2; 2 × CH_3) were observed in the ^{13}C and DEPT NMR spectra of **1** (Table 1). Seven deshielded resonances in the ^1H NMR spectrum [δ_H 6.26, brs (H-17a); 5.30, brs (H-17b); 5.11, brs (H-14); 4.48, brs (H-13); 5.05, overlapped (H-7); 3.65, ABd, J = 10.8 Hz (H-18a); 3.32, ABd, J = 10.8 Hz (H-18b)], two sharp methyl signals [δ_H 0.86, s (H_3-19) and 1.40, s (H_3-20)] (Table 1), and seven deshielded resonances in the ^{13}C NMR spectrum [δ_C 200.8 (s, C-15); 145.6 (s, C-16); 118.0 (t, C-17); 71.3 (t, C-18); 75.7 (d, C-14); 76.8 (d, C-13); 71.9 (d, C-7)] were assigned to a ketone conjugated with an exomethylene group, three oxymethines, one oxymethylene, and two tertiary methyls, by analysis of the corresponding $^1H-^1H$ COSY, HSQC, and HMBC correlations. These observations and the absence of ^{13}C NMR spectroscopic evidence for any further unsaturated functionality indicated the presence of four rings in **1**.

The gross structure (**1a** and **1b**) of **1** was elucidated by analysis of the 2D NMR data including the results of $^1H-^1H$ COSY, HSQC, and HMBC experiments and by comparison with the NMR data of rabdokunmin C.[9] The close similarities of NMR data of the A and B rings with those of known rabdokunmin C suggested a similar structure for these rings. The $^1H-^1H$ COSY correlations of H-1 with H-2, H-2 with H-3, and H-6 with H-7, as well as HMBC correlations of H_3-20 with C-1, C-5, C-9, and C-10, of H_3-19 with C-3, C-4, C-5, and C-18, and of H-7 with C-6, C-8, and C-9, were used to confirm the substructure **1a**. Further comparison of the NMR spectroscopic data of **1** with those of rabdokunmin C revealed that the two compounds differ in their D and E rings. COSY correlations of H-9 with H-11 and of H-11 with a methine proton (H-12), instead of between H-11 and an oxymethine proton, were observed, which suggested in **1** a migrated C-12−C-16 bond rather than a C-13−C-16 bond. This deduction explained all the correlations observed in the HMBC spectrum (Figure 1). In addition, the ^{13}C NMR signal for C-15 shifted upfield from δ_C 209.5 in rabdokunmin C to δ_C 200.8 in **1**, which corroborated the occurrence of the ketone in a six-membered ring rather than a rigid five-membered ring. Two hydroxy groups were located at C-13 and C-14, respectively, on the basis of the HMBC correlations of H-13 with C-8, C-11, and C-16 and of H-14 with C-7 and C-15. Compound **1** was proposed as having a rearranged 16(13→12)-*abeo*-*ent*-kaurane skeleton (*ent*-atisane skeleton).

The relative stereochemistry of compound **1** was established by analyzing information from the ROESY spectrum and proton coupling constants and by comparison of its spectroscopic data with those of rabdokunmin C. The same relative configuration of rings A and B in compound **1** as in rabdokunmin C[9] was deduced from the similar carbon and proton chemical shifts and ROESY correlations found in **1** (Figure 2). Considering that all the kauranoids isolated from the genus *Isodon* possess an *ent*-configuration, **1** was presumed to be an *ent*-atisane diterpenoid. A β-orientation for OH-14 was suggested from the strong ROESY correlations of H-14

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Table 1. ^{1}H and ^{13}C NMR Data for Alboatisins A−C (**1**−**3**)[a] in Pyridine-d_5

	alboatisin A (**1**)		alboatisin B (**2**)		alboatisin C (**3**)	
position	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.
1α	1.54 (m)	39.0 (t)[b]	1.55 (m)	38.2 (t)	1.66 (m)	39.4 (t)
1β	0.72 (m)		0.73 (m)		0.76 (m)	
2α	1.53 (m)	18.2 (t)	1.42 (m)	17.0 (t)	1.53 (m)	18.0 (t)
2β	1.62 (m)		1.57 (m)		1.63 (m)	
3α	1.75 (m)	35.7 (t)	1.30 (m)	32.4 (t)	1.84 (m)	35.8 (t)
3β	1.38 (m)		1.15 (m)		1.42 (m)	
4		37.8 (s)		49.4 (s)		37.9 (s)
5	1.62 (m)	46.0 (d)	1.41 (m)	44.8 (d)	1.64 (m)	46.0 (d)
6α	2.01 (m)	29.0 (t)	1.65 (m)	31.5 (t)	1.85 (m)	27.9 (t)
6β	2.36 (dd, 12.8, 4.1)		2.05 (dd, 12.5, 4.0)		2.26 (dd, 12.5, 4.8)	
7	5.05 (in H_2O)	71.9 (d)	4.88 (in H_2O)	71.2 (d)	4.77 (dd, 11.5, 4.8)	69.8 (d)
8		56.6 (s)		56.8 (s)		51.7 (s)
9	1.46 (m)	48.0 (d)	1.39 (m)	47.7 (d)	1.40 (m)	47.6 (d)
10		39.0 (s)[b]		37.9 (s)		39.2 (s)
11α	1.61 (m)	20.7 (t)	1.64 (m)	20.6 (t)	1.53 (m)	20.7 (t)
11β	2.41 (dd, 12.4, 7.7)		2.38 (dd, 12.4, 7.7)		2.51 (dd, 12.6, 7.7)	
12	3.17 (brs)	43.9 (d)	3.17 (brs)	43.9 (d)	2.99 (brs)	44.8 (d)
13	4.48 (brs)	76.8 (d)	4.45 (brs)	76.7 (d)	4.38 (dd, 10.3, 3.0)	66.7 (d)
14α		75.7 (d)		75.7 (d)	2.39 (dd, 15.5, 3.0)	28.7 (t)
14β	5.11 (brs)		5.00 (brs)		2.73 (dd, 15.5, 10.3)	
15		200.8 (s)		200.3 (s)		202.5 (s)
16		145.6 (s)		145.5 (s)		146.5 (s)
17a	6.26 (brs)	118.0 (t)	6.28 (brs)	118.3 (t)	6.20 (brs)	118.1 (t)
17b	5.30 (brs)		5.20 (brs)		5.25 (brs)	
18a	3.65 (d, 10.8)	71.3 (t)	9.27 (s)	206.0 (d)	3.67 (d, 10.5)	71.4 (t)
18b	3.32 (d, 10.8)				3.33 (d, 10.5)	
19	0.86 (s)	18.1 (q)	1.03 (s)	16.3 (q)	0.86 (s)	18.1 (q)
20	1.40 (s)	16.6 (q)	1.32 (s)	14.4 (q)	1.50 (s)	16.8 (q)

[a] ^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz, and multiplicities inferred from DEPT and HSQC experiments. [b] Overlapped signals.

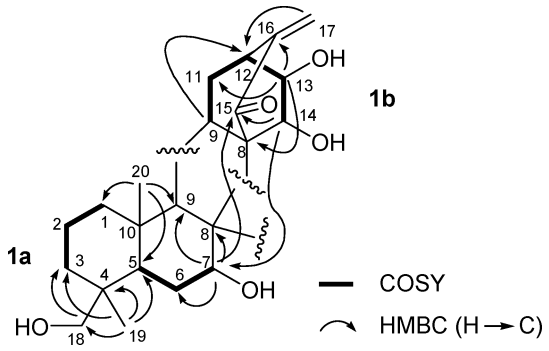

Figure 1. Key HMBC and COSY correlations for **1**.

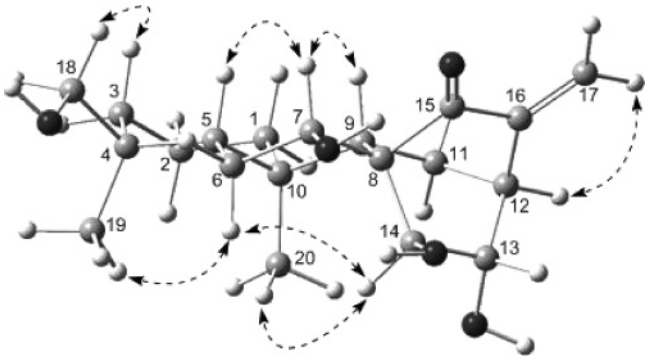

Figure 2. Key ROESY correlations for **1**.

with H_3-20 and H-6α, as shown in a computer-generated 3D model (Figure 2). The relative configuration of OH-13 was inferred as being α, judging from the small coupling constant between H-13 and H-14 ($J \approx 0$ Hz), indicative of the dihedral angle between H-13 and H-14 being near 90°, as shown in Figure 2. If OH-13 is in the β-orientation, the dihedral angle between H-13 and H-14 would be about 0° and the expected coupling constant about 8−10 Hz. Thus, the structure of **1**, named alboatisin A, was determined as 7α,13α,14β,18-tetrahydroxy-*ent*-atis-16-en-15-one.

Alboatisin B (**2**) was assigned the molecular formula $C_{20}H_{28}O_5$, as deduced from positive HRESIMS data (*m*/*z* 371.1839 [M + Na]$^+$). Comparison of the spectroscopic data of **2** with those of **1** revealed many similarities except for the moiety at C-18. Observation of the presence of an aldehyde group (δ_C 206.2, d) and the absence of an oxymethylene carbon in the ^{13}C NMR spectrum of **2** therefore permitted an aldehyde group to be placed at the C-18 position, instead of a hydroxyl group at the same position in **1**. This was confirmed by the downfield chemical shift of C-4 from δ_C 37.8 (s) in **1** to δ_C 49.4 (s) in **2**. Thus, compound **2** was determined structurally as 7α,13α,14β-trihydroxy-*ent*-atis-15-oxo-18-al-16-ene.

Alboatisin C (**3**) was assigned the molecular formula $C_{20}H_{30}O_4$, on the basis of its HRESIMS and NMR data. Comparison of the spectroscopic data of **3** with those of **1** (Table 1) showed a general similarity except that the C-14-oxygenated methine in **1** was replaced by a methylene group (δ_C 28.7, t) in **3**. Examination of its 2D NMR data allowed **3** to be deduced as 7α,13α,18-trihydroxy-*ent*-atis-16-en-15-one.

Compounds **1**−**3** are the first *ent*-atisane diterpenoids reported from the genus *Isodon*. The oxygenated atisanes previously reported are normally diol or triol derivatives, with C-13-oxygenated atisanes being rare among naturally occurring atisane diterpenoids.[10] Two C-13-oxygenated atisanes were synthesized through acid-catalyzed rearrangement of a C-12-oxygenated *ent*-kaurane derivative, in which the oxygenated substituents at C-12 were indispensable for the reaction.[11] Recently, Toyota et al. reported the total synthesis of two atisane diterpenoids (serofendic acids A and B). The key strategic element of the approach involved a similar rearrangement.[12] Therefore, co-occurrence of the C-13-oxygenated atisane diterpenoids (**1**−**3**) and the C-12-oxygenated kaurane diterpenoid rabdokunmin C in the same plant indicates that alboatisins A−C (**1**−**3**) may be biosynthesized by the carbocationic rearrangement of structurally related C-12-oxygenated kauranoids (see Supporting Information).

Compounds **1**−**3** were tested for cytotoxicity against A549, HT-29, and K562 cells using the sulforhodamine B (SRB) method, conducted in a manner reported previously.[13] Compounds **1**−**3**

demonstrated inhibitory activity against A549 (human lung adenocarcinoma cells) (IC_{50} 10.5, 8.3, and 12.5 μM, respectively), HT-29 (human colon cancer cells) (IC_{50} 15.6, 7.9, and 20.6 μM, respectively), and K562 (human lymphocytic leukemia cells) (IC_{50} 24.6, 13.6, and 28.0 μM, respectively).

Experimental Section

General Experimental Procedures. Optical rotations were carried out on a Perkin-Elmer model 241 polarimeter. UV spectra were obtained in a UV 210A spectrometer. IR spectra were measured in a Bio-Rad FTS-135 spectrometer as KBr pellets. 1D and 2D NMR spectra were taken on a Bruker AM-400 or a Bruker DRX-500 NMR spectrometer with TMS as internal standard. Mass spectra were recorded on a VG Auto spec-3000 spectrometer or on a Finnigan MAT 90 instrument. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm × 25 cm, column. Column chromatography was performed on silica gel (200−300 mesh; Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40−63 μm, Merck, Darmstadt, Germany), MCI gel (75−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and Sephadex LH-20 (Pharmacia).

Plant Material. The aerial parts of *I. albopilosus* were collected in Maoxian, Sichuan Province, People's Republic of China, in July 2004. The sample was identified by Prof. Xi-Wen Li, and a voucher specimen (KIB 04092101) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy Sciences.

Extraction and Isolation. Air-dried and powdered aerial parts of *I. albopilosus* (1.1 kg) were extracted with acetone (5 L × 4, each 2 days) at room temperature. After evaporating the solvents in vacuo at 45 °C, a residue (85 g) was obtained. This residue was dissolved in H_2O (1.0 L) and then extracted successively with petroleum ether (60−90 °C, 0.5 L × 2) and EtOAc (1.0 L × 4). The EtOAc extract (35 g) was chromatographed on MCI-gel CHP 20P (90% MeOH−H_2O, 100% MeOH). The 90% MeOH fraction (30 g) was subjected to column chromatography over silica gel (200−300 mesh) and eluted with $CHCl_3$−Me_2CO (from 1:0 to 0:1) to give fractions A−E. Fraction C (11.2 g) was chromatographed on RP-18 eluted with a MeOH−H_2O (30%−100%) gradient system to afford four main fractions, C1−C4. Subfraction C4 (1.2 g) was subjected to silica gel column chromatography, eluted with cyclohexane−isopropyl alcohol (30:1), to give an impure subfraction. This subfraction was further separated by semipreparative HPLC using MeOH−H_2O (45%) as mobile phase (flow rate 3 mL/min) to yield compound **3** (4.3 mg). Fraction D (10.5 g) was divided into subfractions D1−D5 by passage over a RP-18 column, eluted with MeOH−H_2O (from 30% to 100%). Rabdokunmin C (45.0 mg) and **2** (9.0 mg) were obtained from subfraction D2 (1.2 g) by repeated silica gel column chromatography eluted with $CHCl_3$−Me_2CO (8:1) and Sephadex LH-20 chromatography eluted with CH_3OH. Subfraction D3 (0.6 g) was chromatographed on silica gel using cyclohexane−isopropyl alcohol (20:1) as solvent and purified by semipreparative HPLC (MeOH−CH_3CN−H_2O, 45:5:50) to yield compound **1** (12 mg).

Alboatisin A (1): white powder; $[\alpha]_D^{19}$ −18.6 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 236 (4.22) nm; IR (KBr) ν_{max} 3424, 2925, 2872, 1724, 1711, 1632, 1464, 1444, 1390, 1342, 1304, 1152, 1052, 1037 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 350 (9), 332 (22), 321 (43), 301 (21), 275 (26), 109 (100), 193 (27); HRESIMS (positive ion) *m*/*z* 373.1984 (calcd for $C_{20}H_{30}O_5Na$ $[M + Na]^+$, 373.1990).

Alboatisin B (2): white powder; $[\alpha]_D^{19}$ −16.7 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ϵ) 234 (4.32) nm; IR (KBr) ν_{max} 3452, 2933, 2854, 1727, 1706, 1632, 1444, 1397, 1385, 1099, 1063, 1036 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 348 (8), 330 (33), 319 (68), 301 (24), 273 (61), 213 (22), 109 (100); HRESIMS (positive ion) *m*/*z* 371.1839 (calcd for $C_{20}H_{28}O_5Na$ $[M + Na]^+$, 371.1834).

Alboatisin C (3): white powder; $[\alpha]_D^{19}$ −34.7 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 234 (4.30) nm; IR (KBr) ν_{max} 3448, 2930, 2858, 1724, 1710, 1632, 1444, 1398, 1387, 1066, 1046 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 334 (6), 328 (23), 317 (48), 301 (25), 259 (55), 213 (22), 91 (100); HRESIMS (positive ion) *m*/*z* 357.2038 (calcd for $C_{20}H_{30}O_4Na$ $[M + Na]^+$, 357.2042).

Cytotoxicity Testing. Cytotoxicity of compounds against suspended tumor cells was determined by the trypan blue exclusion method and against adherent cells by sulforhodamine B (SRB) assay. Cells were plated in a 96-well plate 24 h before treatment and continuously exposed to different concentrations of compounds for 72 h. After compound treatment, cells were counted (suspended cells) or fixed and stained with SRB (adherent cells) as described in the literature.[13] Amrubicin hydrochloride was used as a positive control with IC_{50} values of 0.82 (A549), 4.36 (HT-29), and 1.26 μM (K562), respectively.

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Supporting Information Available: The NMR (1D and 2D) and mass spectra of compounds **1**−**3**, NMR data for rabdokunmin C, and proposed biosynthetic pathway for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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